FEBS Letters 587 (2013) 1399–1404

FEBS Letters

journal homepage: www.FEBSLetters.org

The *Trypanosoma brucei* telomerase RNA (TER) homologue binds core proteins of the C/D snoRNA family

Sachin Kumar Gupta [a,b,1], Liat Kolet [a,b,1], Tirza Doniger [a,b], Viplov Kumar Biswas [a,b], Ron Unger [a,b], Yehuda Tzfati [c], Shulamit Michaeli [a,b,*]

[a] *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*
[b] *Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan 52900, Israel*
[c] *Department of Genetics, The Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel*

ARTICLE INFO

Article history:
Received 1 January 2013
Revised 6 March 2013
Accepted 7 March 2013
Available online 21 March 2013

Edited by Varda Rotter

Keywords:
Telomerase
TER RNA
C/D snoRNA
Trans-splicing of non-coding RNAs
Trypanosoma brucei

ABSTRACT

Trypanosome protozoan parasites are the causative agents of devastating diseases. Trypanosome telomeres grow in an uncontrolled manner and the variant surface glycoprotein (VSG) genes are located in subtelomeric domains. The gene encoding telomerase reverse transcriptase (TERT) was identified and in this study, we describe the *Trypanosoma brucei* telomerase RNA (TER). TER RNA is bound by the core proteins of the C/D small nucleolar RNA (snoRNA) family and associates with the methyltransferase-associated protein (MTAP), whose homologue binds to mammalian TER. Silencing of *Tb*TER resulted in telomere shortening. This is the first report of a TER that binds the C/D snoRNA core proteins.

1. Introduction

Telomerases from all species contain a RNA component (telomerase RNA, TER), and a unique reverse transcriptase protein (telomerase reverse transcriptase, TERT), as well as additional proteins, which differ among eukaryotes, and function in assembly, localization and linking of telomerase to other cellular functions [1]. The function of telomerase was first described in the ciliated protozoa, *Tetrahymena thermophila*. It was demonstrated that the telomere sequence TTGGG is synthesized on an RNA template present in TER [2–4]. In vertebrates, telomerase activity is low in most somatic cells, but is highly expressed in proliferative cells including cancer cells [5–7].

Telomerase RNAs (TERs) are highly divergent among the different species, varying in both size and sequence composition, ranging from 150 nt in ciliates to 450 nt in vertebrates and 930–1300 nt in the budding yeasts *Kluyveromyces* and *Sacchaomyces* [8]. All TERs contain a 5′ template boundary element (TBE) and a large loop that includes the template, a potential pseudoknot and a loop-closing helical region. In vertebrates, this core domain is required for telomerase activity and can be combined, in *trans* with the 3′ end domain of the RNA, and the telomerase reverse transcriptase (TERT) to reconstitute telomerase activity in vitro [9]. Yeast TER (TLC1) is the longest TER described so far. The core domain containing the putative pseudoknot was shown to bind TERT (Est2p) [10] and Ku and Sm, which are the proteins that stabilize the RNA [11].

Human telomerase (hTR) is associated with the complete set of box H/ACA RNP proteins [12]. The 3′ terminal box H/ACA acts to stabilize the RNP and is also essential for telomerase function [13]. The H/ACA domain determines the correct intracellular localization [14]. The apical loop of the 3′ terminal hairpin of hTR carries a Cajal body (CB) localization signal, the CAB box, which is also present in a subclass of box H/ACA pseudouridylation-guide RNAs that accumulate in CBs and are called small CB-specific RNAs (scaRNA) [15]. Indeed, the TCAB1 protein or WDR79 was shown to bind to scaRNA and telomerase RNAs and to determine their localization to the CBs [16]. This protein was found to be related to the trypanosome protein that binds to the spliced leader associated RNA (SLA1); it guides pseudouridylation on the spliced leader (SL) RNA [17,18]. This SLA1 binding protein was termed MTAP [19].

Trypanosome protozoan parasites are the causative agent of devastating diseases in humans and livestock and diverged very early from the eukaryotic lineage and are famous for the discovery of basic and unique molecular mechanisms in the RNA world, such

* Corresponding author at: The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel. Fax: +972 3 7384058.
E-mail address: shulamit.michaeli@biu.ac.il (S. Michaeli).
[1] The first two authors contributed equally to this work.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.03.017

as *trans*-splicing [17]. Trypanosome telomeres were found to possess several unusual characteristics, such as uncontrolled telomere growth and occupation of sub-telomeric regions by variant surface glycoprotein (VSG) genes [20]. In *Trypanosoma brucei* and *Trypanosoma cruzi*, the termini share the human consensus sequence 5′-GGGTTAGGG-3′ [21]. The gene encoding TERT activity was identified in trypanosomatids [22,23]. Knock-out of TERT results in shorter and fragile telomeres. After long term culture, most mini-chromosomes disappear, and some intermediate chromosomes rearrange. Since depletion of TERT is not lethal, it was suggested that a telomerase-independent mechanism is responsible for the stabilization of mega and intermediate chromosomes [24,25].

Despite the extensive studies on *T. brucei* telomerase, the RNA component of the enzyme complex has not been identified. In this study, we identify the telomerase RNA as a ~1 kb species. *Tb*TER RNA is bound by the core proteins of C/D snoRNAs and MTAP, which in humans binds TER and scaRNAs. Like trypanosome snoRNAs, TER is processed from precursors that are *trans*-spliced and polyadenylated [26]. *In situ* hybridization demonstrates that *Tb*TER co-localizes with TERT outside the nucleolus. TER is a bona-fide C/D RNA as its expression is destabilized in cells silenced for NOP58, and it stably associates with SNU13. Silencing of *Tb*TER by RNAi results in telomere shortening. This is the first report of a TER that binds the C/D snoRNA core proteins.

2. Materials and methods

The oligonucleotides used in this study are listed in S-1.

2.1. Cell growth, construct preparation and transfection

Procyclic forms of *T. brucei* strain 29–13 were grown and transfected as previously described [27]. The silencing constructs were prepared as in [28] and the PTP-tagged *T. brucei* TERT were as described in [29].

2.2. Identification of TER RNA

Affinity-chromatography was as described [30] and library construction for Illumina sequencing was as previously described and details for the protocol is given in S-2 [31]. The reads were viewed by the Integrative Genomics Viewer (IGV) at http://www.broadinstitute.org/igv/.

2.3. Southern analyses

Cell carrying the telomerase silencing constructs were subcultured every second day in the tetracycline-containing medium. Genomic DNA was isolated at intervals of ~1 week digested with Sau3AI and AluI. The restricted DNA samples (10 μg) were separated on a 1.5% agarose gel and blotted. Terminal restriction fragments were visualized using a radiolabeled $(TTAGGG)_4$ oligonucleotide probe.

2.4. In situ hybridization combined with immunofluorescence

In situ hybridization to localize *Tb*TER was performed as described [18,32]. Nuclei were stained using 4′-6′-diamidino-2-phenylindole (DAPI). The cells were visualized under a Zeiss LSM 510 META inverted microscope.

2.5. RT-PCR

RNA was extensively treated with the DNase-based DNA inactivation reagent (DNA-free; Ambion, AM 1906) to remove the DNA contamination. cDNA was prepared as previously reported [33,34] and amplified with the primers listed in S-1.

3. Results

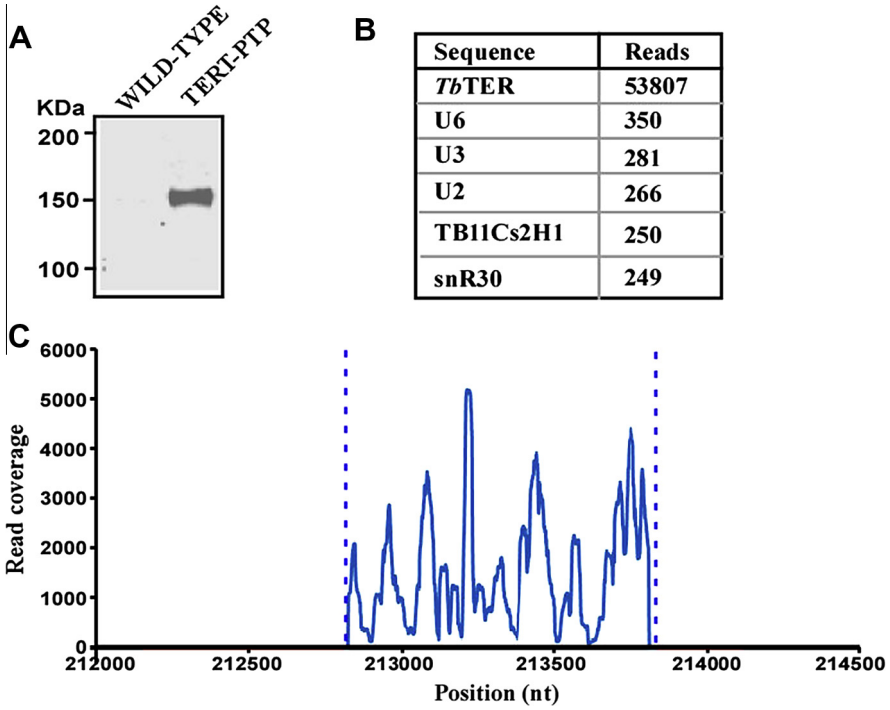
We recently described the repertoire of small RNAs in *T. brucei* [31]; in this library, we identified low-abundance reads of a RNA fragment containing the sequence 5′-CCCUAACCC-3′, complementary to the telomere sequence. We suspected that this sequence could represent the trypanosome TER.

To verify that this sequence is indeed the *Tb*TER, one allele of the *Tb*TERT locus was tagged with the TAP-PTP epitope [30]. The expression of the tagged protein is presented in Fig. 1A. A whole cell extract from this cell line was used to affinity select the RNA associated with TERT, and a library was prepared from the affinity-selected RNA. The library was enriched for *Tb*TER, as can be seen from the number of reads obtained for *Tb*TER as compared to other abundant small RNA species (Fig. 1B and C). The RNA-seq data enabled us to determine the precise sequence at the 5′ and 3′ boundaries of the RNA, revealing a transcript of 993 nt (presented in S-3).

The sequence of *Tb*TER was used to search for sequences of other *Trypanosoma* species using the BLAST algorithm [35], and homologues with significant sequence identity were identified in the genomes of *Trypanosoma cruzi*, *Trypanosoma vivax*, and *Trypanosoma congolense*. Importantly, all four candidate TERs contain the expected template sequence. The alignment of all four sequences is shown in S-4. An attempt was made to obtain a secondary structure of the molecule using the four sequences and the software *RNAalifold*, which combines free energy calculations and phylogenetic co-variation [36]. Although we could not propose a secondary structure model for the entire RNA, *RNAalifold* predicted several phylogenetically-supported helices, particularly in the templating domain and the box C/D domain, which were used to constrain *MFOLD* to predict secondary structure models for these domains of *Tb*TER (Fig. 2A and B). In the templating domain, the template sequence is predicted mostly single-stranded, as expected from a sequence that serves as a template for DNA synthesis. Two additional elements are conserved with secondary structure predictions in other species: (1) A highly-stable helix located upstream of the template sequence, presumably serving as a template boundary element as found in other organisms [37] (TBE; Fig. 2A); and (2) a highly conserved sequence (CS1) located at the base of the TBE in the four trypanosomatid TERs (Fig. 2A), similarly to other conserved sequences found in the same location in Yeast TERs [10]. The presence of a conserved sequence in such a close proximity to the template suggests that it plays a role in telomere repeat synthesis. Close to the 3′ end of the RNA, we identified another conserved sequence that we termed CS2 (S-4). We assume that the sequence conservation of CS2 reflects a conserved function, as shown for conserved sequences in other TERs, however, we do not know yet what this conserved function might be.

Interestingly, at the 5′ and 3′ ends of *Tb*TER we identified boxes C and D consensus sequences, which are predicted to partially pair with each other. These sequences are conserved in all four trypanosomatid TERs and may specify TER as a C/D snoRNA (Fig. 2B).

In yeast, TER RNA is associated with Sm core proteins, whereas mammalian TER is an H/ACA RNP [8]. To investigate the core proteins that bind to *Tb*TER, and especially, to examine if *Tb*TER belongs to the C/D snoRNA family, the level of *Tb*TER was analyzed by Northern analysis following silencing of CBF5 (a H/ACA snoRNA core protein), NOP58 (a C/D snoRNA protein) [38,39], SmD1, which binds to U1, U2, U4, and U5 snRNAs [40], and Lsm3, which binds to U6 snRNA [41,42]. The results (Fig. 3A) demonstrate that the *Tb*TER level is reduced only upon NOP58 silencing, suggesting that *Tb*TER



Sequence	Reads
*Tb*TER	53807
U6	350
U3	281
U2	266
TB11Cs2H1	250
snR30	249

Fig. 1. Identification of the telomerase RNA (TER) sequence in *T. brucei*. (A) Western analysis demonstrating the expression of TERT-PTP. TERT was PTP-tagged and proteins were extracted from 10^7 cells, and subjected to western analysis with rabbit IgG (Sigma). Wild-type cells were used as a negative control for cross-reacting proteins. (B) Annotation of the reads obtained from the TERT affinity-RNA-seq library. The identity or annotation of the sequence obtained from the TERT affinity-RNA-seq library along with their reads is given. (C) IGV viewer representation of the TER in the TERT-RNA-seq library. A library was prepared from the affinity selected RNA after mild alkali hydrolysis. The reads of the TER sequence is given in blue. 5′ (Tb927_11_01_v4, sequence: 212820) and 3′ (Tb927_11_01_v4, sequence: 213812) boundaries of the TER are shown by blue dashed vertical lines and the exact sequence is presented in S-3.

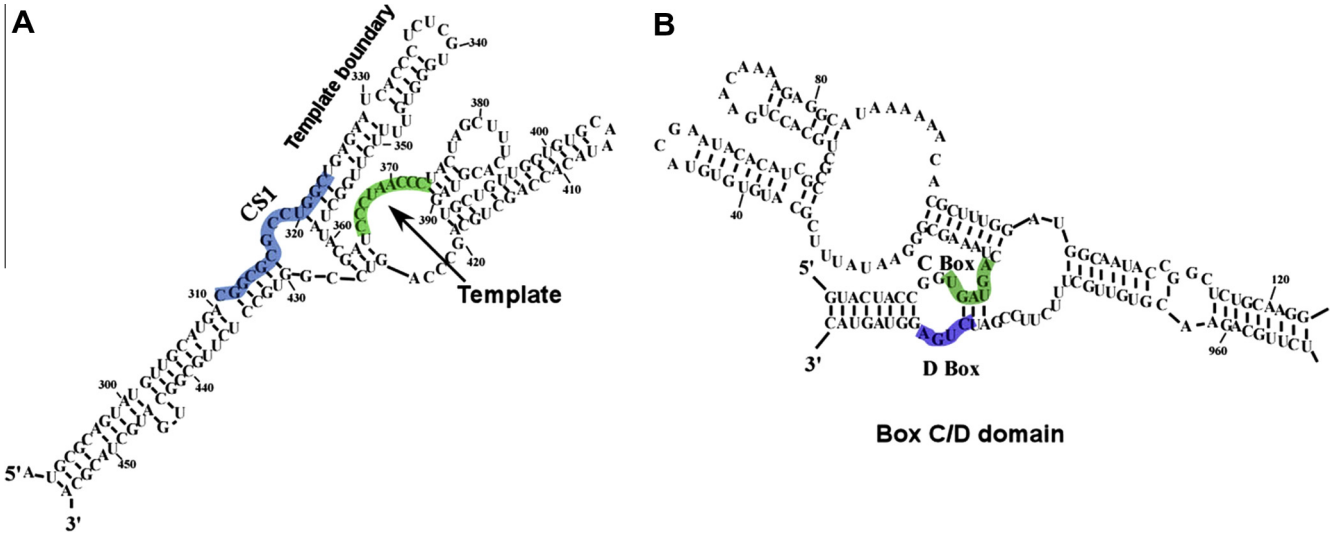


Fig. 2. Secondary structure models of the templating domain and the box C/D domain of TER. The prediction of two domains is strongly supported by phylogenetic covariations (S-4). (A) The templating domain and (B) the box C/D domain. The structures were predicted by *MFOLD* [47], using constraints derived from the *RNAalifold* prediction [36].

is indeed bound by C/D snoRNA core proteins. Next, we examined if *Tb*TER can be specifically affinity-selected by C/D core proteins. To this end, we used a *T. brucei* cell lines TAP-tagged with the following proteins; SNU13, which binds C/D snoRNA, NHP2, which binds H/ACA snoRNAs [38], and TERT (generated in this study, [24]). The affinity-selected RNA was analyzed by RT-PCR using primers for an H/ACA RNA (TB11C2H1, or SLA1), a C/D snoRNA (TB11Cs2C1) and *TbTER*. The results (Fig. 3B) demonstrate the selectivity of the affinity purification using tagged TERT and SNU13 for the pull-downs since very efficient selection of TER was obtained only with SNU13 and TERT and not with NHP2.

In mammalian cells, MTAP homologue binds to scaRNAs and TER [16]. We therefore examined if *Tb*TER is associated with MTAP. MTAP was silenced using the T7 opposing promoter system [28]. The levels of *Tb*TER and SLA1 which was shown to bind MTAP [19] were examined under silencing. The results demonstrate that

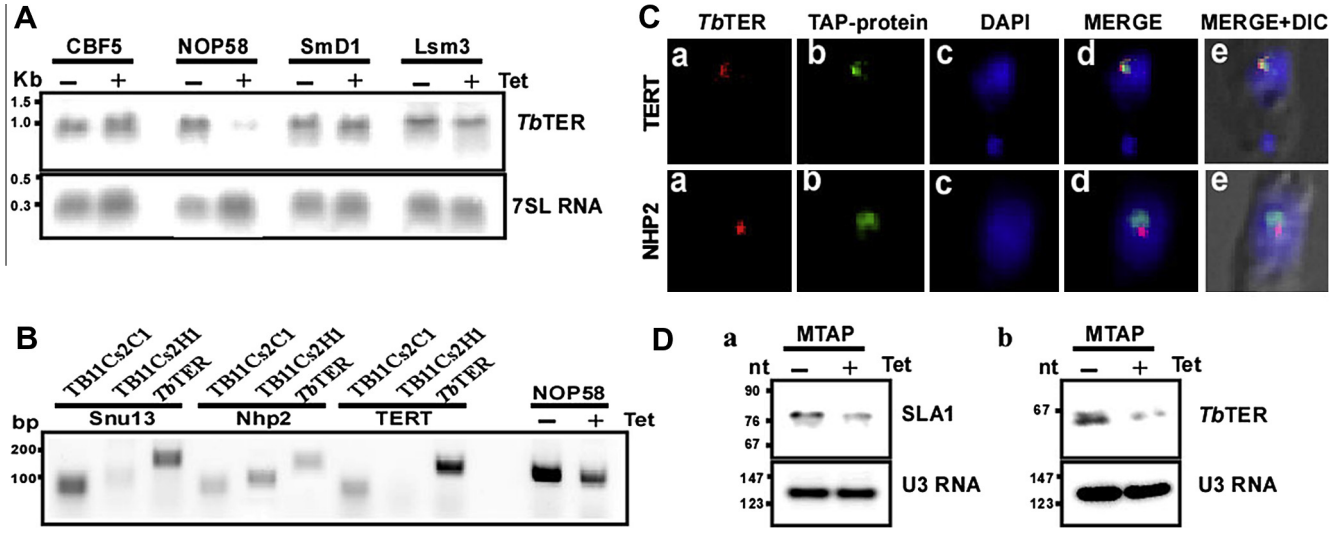


Fig. 3. The *Tb*TER RNP and its localization. (A) *Tb*TER is bound by C/D core proteins. RNA was prepared from uninduced cells, and cells after 3 days of silencing for the factors indicated. Total RNA (20 μg) was separated on a 1.2% agarose/2.2 M formaldehyde gel and the blot was probed with TER antisense RNA probe. 7SL RNA used to control for equal loading. (B) Affinity selection of *TbTER* by epitope tagged TERT and SNU13. RNA was affinity selected from extracts of cells expressing the following TAP-PTP-tagged proteins: SNU13, NHP2 and TERT as described in Section 2, and was subjected to RT-PCR with the indicated gene primers. (Right panel) RNA from NOP58 silenced cells was subjected to silencing for 2.5 days. RT-PCR with primers specific to TER was used to amplify the RNA. (C) Co-localization of *Tb*TER with TERT. *In situ* hybridization combined with immunofluorescence was performed. (a) *In situ* hybridization with a *Tb*TER probe monitored with Alexa-Red; (b) the tagged protein detected with FITC-IgG; (c) DAPI staining; (d) merge of a with c; (e) DIC merged with d. The same analysis was performed for NHP2 but using antibodies to the protein. (D) *Tb*TER binds MTAP. RNA was prepared from cells carrying a MTAP silencing construct, either uninduced or after 2.5 days of silencing. Total RNA (10 μg) was subjected to primer extension with an oligonucleotide complementary to the indicated RNAs. (a) SLA1, (b) *Tb*TER. Primer extension of U3 snRNA was used as a control. The products were separated on a 6% acrylamide denaturing gel.

the level of *Tb*TER, like SLA1 that served as positive control for silencing, was reduced upon MTAP silencing, thus demonstrating that *Tb*TER is bound by MTAP (Fig. 3D).

As an additional criteria for TER being the telomerase we examined the localization of *Tb*TER with respect to *Tb*TERT. The results (Fig. 3C) demonstrate co-localization with *Tb*TERT outside the nucleolus that was stained with NHP2 antibodies.

Since snoRNAs were found to be processed from precursors that are *trans*-spliced and polyadenylated [26], we performed RT-PCR using the SL forward primer and a *Tb*TER internal reverse primer to identify the 5′ end of the *Tb*TER precursor. The results (Fig. 4) show that the SL addition site is located 273 nt upstream from the first nt of *Tb*TER, suggesting that TER is processed from a transcript that is *trans*-spliced.

To further gain support that *Tb*TER is indeed the telomerase RNA, we examined if TER depletion affects telomere length, as in *Tb*TERT null mutant cells [24,25]. *Tb*TER was silenced by RNAi using a stem loop silencing construct whose expression is induced by tetracycline [28]. RNA extracted from cells before and after 2 days of silencing was subjected to Northern analysis, and the results (Fig. 5A) demonstrate efficient silencing. The cell line was then induced for 9 weeks in the presence of tetracycline. The Southern analysis (Fig. 5B) demonstrates shortening of telomeres, further establishing *Tb*TER as the telomerase RNA.

4. Discussion

This study identified the *T. brucei* TER based on: (1) its association with TERT, which enabled its sequencing to determine the exact boundaries of the mature RNA species; (2) its co-localization with TERT in a distinct focus in the nucleus near the nucleolus; and (3) the effect of its silencing, which led to chromosome shortening.

Fig. 4. *Tb*TER undergoes *trans*-splicing. RT-PCR followed by sequencing. (A) cDNA was prepared from wild-type *T. brucei* cells. The pre-TER sequence was amplified using a sense SL RNA primer and an internal reverse primer from the TER sequence. (B) The location of the primers with respect to the *Tb* TER sequence is illustrated. (C) The sequence of the amplified PCR product.

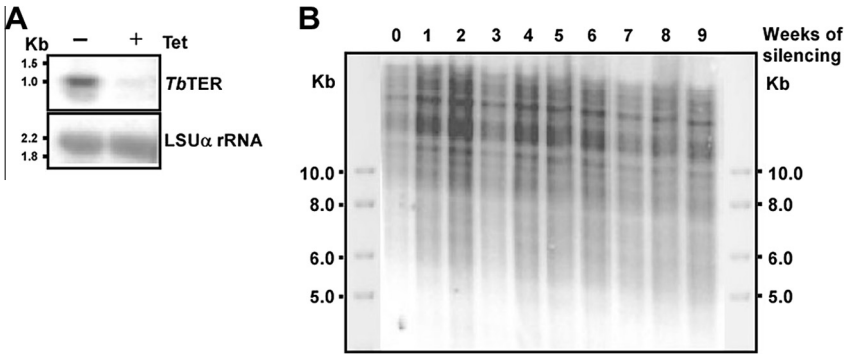


Fig. 5. Telomere shortening during *TbTER* silencing. (A) Silencing *Tb*TER. RNA was prepared from uninduced cells, and cells after 2 days of silencing. Total RNA (20 μg) was separated on a 1.2% agarose/2.2 M formaldehyde gel. The RNA was blotted and hybridized with a T7 transcribed *Tb*TER antisense RNA probe. To control for equal loading, the blot was hybridized with a anti-sense rRNA (LSUα) oligonucleotide. (B) Telomere shortening upon silencing of *Tb*TER. Genomic DNA was isolated from uninduced cells and cells after silencing of *Tb*TER at intervals of ~1 week for a total of 9 weeks as described in Section 2. The genomic DNA was then digested with Sau3AI and AluI. The restricted DNA samples were separated on a 1.5% agarose gel with 1 kb marker in first and last well and blotted. Terminal restriction fragments were visualized, using a radiolabeled $(TTAGGG)_4$ oligonucleotide probe.

The most surprising finding is that *Tb*TER binds the C/D snoRNA core protein. Interestingly, the size of the *Tb*TER, 993 nt, resembles that of the short yeast homologue. However, the lack of additional trypanosomatid TER sequences precludes our ability to suggest a secondary structure for the entire molecule. Over the years it became evident that the size variation of TER resulted from acquisition and deletion of a variety of species-specific structural domains that serve to bind species-specific telomerase-associated proteins. These proteins have distinct functions in telomerase RNP biogenesis *in vivo*, but are not essential for the enzymatic activity *in vitro*, and some domains of TER are even dispensable both *in vivo* and *in vitro* [43]. We anticipate that like other TERs, the trypanosome RNA binds other proteins in addition to TERT and MTAP identified in this study. One such protein is most probably KU80 that was shown to bind to *Saccharomyces cerevisiae* and *Arabidopsis thaliana* TERs, and its depletion in *T. brucei* leads to telomere shortening [44]. The most intriguing question is why *Tb*TER is a C/D snoRNA and not an H/ACA RNA as in vertebrates, an snRNA as in yeast, or a La protein associated RNA as in ciliates (reviewed in [1]). Although we have no concrete answer to this question, it raises other questions regarding the assembly and regulation of *T. brucei* snoRNAs with functions other than RNA modification. The need to assemble a large amount of SLA1 RNA with H/ACA core proteins, may have shifted telomerase to the C/D family to avoid competition with SLA1.

A unique property of *T. brucei* is that their telomeres grow indefinitely in culture, maybe because trypanosomes lack TRF1 that controls telomere growth [45]. Telomeres adjacent to the VSG expression site undergo frequent truncation, counterbalancing telomere elongation by telomerase [20]. Under prolonged growth in the laboratory, antigenic variation switching is diminished, perhaps leading to average telomere elongation [46]. It will be interesting to find out how the lack of negative regulation is reflected in the structure–function of *Tb*TER.

The finding that the TER precursor is *trans*-spliced highlights the importance of this process not only for the generation of all mRNAs but also for the generation of telomerase RNA. Thus, gene expression in trypanosomes links mRNA processing not only to polyadenylation but also to the processing of small non-coding RNAs.

Acknowledgments

This work was supported by a grant from the Israel–US Binational Science Foundation (BSF) to S.M. Who holds the David and Inez Myers Chair in RNA silencing of diseases and another BSF grant to Y.T. We thank Dr. Itai Tkacz for the help in RNA-seq.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.03.017.

References

[1] Collins, K. (2011) Single-stranded DNA repeat synthesis by telomerase. Curr. Opin. Chem. Biol. 15, 643–648.
[2] Greider, C.W. and Blackburn, E.H. (1985) Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell 43, 405–413.
[3] Greider, C.W. and Blackburn, E.H. (1987) The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51, 887–898.
[4] Greider, C.W. and Blackburn, E.H. (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature 337, 331–337.
[5] Greider, C.W. and Blackburn, E.H. (1996) Telomeres, telomerase and cancer. Sci. Am. 274, 92–97.
[6] Kim, N.W. et al. (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266, 2011–2015.
[7] Shay, J.W. and Bacchetti, S. (1997) A survey of telomerase activity in human cancer. Eur. J. Cancer 33, 787–791.
[8] Egan, E.D. and Collins, K. (2012) Biogenesis of telomerase ribonucleoproteins. RNA 18, 1747–1759.
[9] Theimer, C.A. and Feigon, J. (2006) Structure and function of telomerase RNA. Curr. Opin. Struct. Biol. 16, 307–318.
[10] Tzfati, Y., Knight, Z., Roy, J. and Blackburn, E.H. (2003) A novel pseudoknot element is essential for the action of a yeast telomerase. Genes Dev. 17, 1779–1788.
[11] Lebo, K.J. and Zappulla, D.C. (2012) Stiffened yeast telomerase RNA supports RNP function in vitro and in vivo. RNA 18, 1666–1678.
[12] Meier, U.T. (2005) The many facets of H/ACA ribonucleoproteins. Chromosoma 114, 1–14.
[13] Mitchell, J.R. and Collins, K. (2000) Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase. Mol. Cell 6, 361–371.
[14] Jady, B.E., Bertrand, E. and Kiss, T. (2004) Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. J. Cell Biol. 164, 647–652.
[15] Richard, P., Darzacq, X., Bertrand, E., Jady, B.E., Verheggen, C. and Kiss, T. (2003) A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. EMBO J. 22, 4283–4293.
[16] Tycowski, K.T., Shu, M.D., Kukoyi, A. and Steitz, J.A. (2009) A conserved WD40 protein binds the Cajal body localization signal of scaRNP particles. Mol. Cell 34, 47–57.
[17] Michaeli, S. (2011) Trans-splicing in trypanosomes: machinery and its impact on the parasite transcriptome. Future Microbiol. 6, 459–474.
[18] Hury, A., Goldshmidt, H., Tkacz, I.D. and Michaeli, S. (2009) Trypanosome spliced-leader-associated RNA (SLA1) localization and implications for spliced-leader RNA biogenesis. Eukaryot. Cell 8, 56–68.

[19] Zamudio, J.R., Mittra, B., Chattopadhyay, A., Wohlschlegel, J.A., Sturm, N.R. and Campbell, D.A. (2009) *Trypanosoma brucei* spliced leader RNA maturation by the cap 1,2′-O-ribose methyltransferase and SLA1 H/ACA snoRNA pseudouridine synthase complex. Mol. Cell. Biol. 29, 1202–1211.
[20] Lira, C.B., Giardini, M.A., Neto, J.L., Conte, F.F. and Cano, M.I. (2007) Telomere biology of trypanosomatids: beginning to answer some questions. Trends Parasitol. 23, 357–362.
[21] Cano, M.I. (2001) Telomere biology of trypanosomatids: more questions than answers. Trends Parasitol. 17, 425–429.
[22] Cano, M.I., Dungan, J.M., Agabian, N. and Blackburn, E.H. (1999) Telomerase in kinetoplastid parasitic protozoa. Proc. Natl. Acad. Sci. USA 96, 3616–3621.
[23] Munoz, D.P. and Collins, K. (2004) Biochemical properties of *Trypanosoma cruzi* telomerase. Nucleic Acids Res. 32, 5214–5222.
[24] Dreesen, O., Li, B. and Cross, G.A. (2005) Telomere structure and shortening in telomerase-deficient *Trypanosoma brucei*. Nucleic Acids Res. 33, 4536–4543.
[25] Dreesen, O. and Cross, G.A. (2006) Telomerase-independent stabilization of short telomeres in *Trypanosoma brucei*. Mol. Cell. Biol. 26, 4911–4919.
[26] Kolev, N.G., Franklin, J.B., Carmi, S., Shi, H., Michaeli, S. and Tschudi, C. (2010) The transcriptome of the human pathogen *Trypanosoma brucei* at single-nucleotide resolution. PLoS Pathog. 6, e1001090.
[27] Mandelboim, M., Barth, S., Biton, M., Liang, X.H. and Michaeli, S. (2003) Silencing of Sm proteins in *Trypanosoma brucei* by RNA interference captured a novel cytoplasmic intermediate in spliced leader RNA biogenesis. J. Biol. Chem. 278, 51469–51478.
[28] Wang, Z., Morris, J.C., Drew, M.E. and Englund, P.T. (2000) Inhibition of *Trypanosoma brucei* gene expression by RNA interference using an integratable vector with opposing T7 promoters. J. Biol. Chem. 275, 40174–40179.
[29] Palfi, Z., Schimanski, B., Gunzl, A., Lucke, S. and Bindereif, A. (2005) U1 small nuclear RNP from *Trypanosoma brucei*: a minimal U1 snRNA with unusual protein components. Nucleic Acids Res. 33, 2493–2503.
[30] Schimanski, B., Nguyen, T.N. and Gunzl, A. (2005) Highly efficient tandem affinity purification of trypanosome protein complexes based on a novel epitope combination. Eukaryot. Cell 4, 1942–1950.
[31] Michaeli, S. et al. (2012) RNA-seq analysis of small RNPs in *Trypanosoma brucei* reveals a rich repertoire of non-coding RNAs. Nucleic Acids Res. 40, 1282–1298.
[32] Tkacz, I.D., Gupta, S.K., Volkov, V., Romano, M., Haham, T., Tulinski, P., Lebenthal, I. and Michaeli, S. (2010) Analysis of spliceosomal proteins in Trypanosomatids reveals novel functions in mRNA processing. J. Biol. Chem. 285, 27982–27999.
[33] Gupta, S.K., Hury, A., Ziporen, Y., Shi, H., Ullu, E. and Michaeli, S. (2010) Small nucleolar RNA interference in *Trypanosoma brucei*: mechanism and utilization for elucidating the function of snoRNAs. Nucleic Acids Res. 38, 7236–7247.
[34] Stern, M.Z. et al. (2009) Multiple roles for polypyrimidine tract binding (PTB) proteins in trypanosome RNA metabolism. RNA 15, 648–665.
[35] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
[36] Hofacker, I.L., Fekete, M. and Stadler, P.F. (2002) Secondary structure prediction for aligned RNA sequences. J. Mol. Biol. 319, 1059–1066.
[37] Tzfati, Y., Fulton, T.B., Roy, J. and Blackburn, E.H. (2000) Template boundary in a yeast telomerase specified by RNA structure. Science 288, 863–867.
[38] Barth, S. et al. (2008) Elucidating the role of C/D snoRNA in rRNA processing and modification in *Trypanosoma brucei*. Eukaryot. Cell 7, 86–101.
[39] Barth, S., Hury, A., Liang, X.H. and Michaeli, S. (2005) Elucidating the role of H/ACA-like RNAs in trans-splicing and rRNA processing via RNA interference silencing of the *Trypanosoma brucei* CBF5 pseudouridine synthase. J. Biol. Chem. 280, 34558–34568.
[40] Tkacz, I.D., Lustig, Y., Stern, M.Z., Biton, M., Salmon-Divon, M., Das, A., Bellofatto, V. and Michaeli, S. (2007) Identification of novel snRNA-specific Sm proteins that bind selectively to U2 and U4 snRNAs in *Trypanosoma brucei*. RNA 13, 30–43.
[41] Tkacz, I.D., Cohen, S., Salmon-Divon, M. and Michaeli, S. (2008) Identification of the heptameric Lsm complex that binds U6 snRNA in *Trypanosoma brucei*. Mol. Biochem. Parasitol. 160, 22–31.
[42] Liang, X.H., Haritan, A., Uliel, S. and Michaeli, S. (2003) trans and cis splicing in trypanosomatids: mechanism, factors, and regulation. Eukaryot. Cell 2, 830–840.
[43] Zappulla, D.C., Goodrich, K. and Cech, T.R. (2005) A miniature yeast telomerase RNA functions in vivo and reconstitutes activity in vitro. Nat. Struct. Mol. Biol. 12, 1072–1077.
[44] Janzen, C.J., Lander, F., Dreesen, O. and Cross, G.A. (2004) Telomere length regulation and transcriptional silencing in KU80-deficient *Trypanosoma brucei*. Nucleic Acids Res. 32, 6575–6584.
[45] Bernards, A., Michels, P.A., Lincke, C.R. and Borst, P. (1983) Growth of chromosome ends in multiplying trypanosomes. Nature 303, 592–597.
[46] Hovel-Miner, G.A., Boothroyd, C.E., Mugnier, M., Dreesen, O., Cross, G.A. and Papavasiliou, F.N. (2012) Telomere length affects the frequency and mechanism of antigenic variation in *Trypanosoma brucei*. PLoS Pathog. 8, e1002900.
[47] Zuker, M. (2003) *MFOLD* web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31, 3406–3415.